Journal of Neuroscience Research 69:966–975 (2002)

Progenitor Cells Derived From the Adult Human Subcortical White Matter Disperse and Differentiate as Oligodendrocytes Within Demyelinated Lesions of the Rat Brain

Martha S. Windrem,[1] Neeta S. Roy,[1] Jeremy Wang,[2] Marta Nunes,[1] Abdellatif Benraiss,[1] Robert Goodman,[3] Guy M. McKhann II,[3] and Steven A. Goldman[1*]

[1]Department of Neurology and Neuroscience, Cornell University Medical College, New York, New York
[2]Department of Neurosurgery, Cornell University Medical College, New York, New York
[3]Columbia College of Physicians and Surgeons, New York, New York

A distinct population of white matter progenitor cells (WMPCs), competent but not committed to generate oligod ndrocytes, remains ubiquitous in the adult human subcortical white matter. These cells are present in both sexes and into senescence and may constitute as much as 4% of the cells of adult human capsular white matter. Transduction of adult human white matter dissociates with plasmids bearing early oligodendrocytic promoters driving fluorescent reporters permits the separation of these cells at high yield and purity, as does separation based on their expression of A2B5 immunoreactivity. Isolates of these cells survive xenograft to lysolecithin-demyelinated brain and migrate rapidly to infiltrate these lesions, without extending into normal white matter. Within several weeks, implanted progenitors mature as oligodendrocytes, and develop myelin-associated antigens. Lentiviral tagging with green fluorescent protein confirmed that A2B5-sorted progenitors develop myelin basic protein expression within regions of demyelination and that they fail to migrate when implanted into normal brain. Adult human white matter progenitor cells can thus disperse widely through regions of experimental demyelination and are able to differentiate as myelinating oligodendrocytes. This being the case, they may constitute appropriate vectors for cell-based remyelination strategies.

K y words: transplant; remyelination; myelin; precursor cells; neural stem cells; cell sorting

Glial progenitor cells biased toward oligodendrocytic production persist within the adult human white matter and may be isolated and propagated as such (for review see Goldman, 2001). These cells appeared to correspond, in both function and phenotype, to analogous progenitors in the adult rodent white matter, whose phenotypic potential had been previously established in vitro (Wolswijk and Noble, 1989) and validated by retroviral lineage analysis in vivo (Gensert and Goldman, 1996, 1997; Levison and Goldman, 1999). The existence of postmitotic pro-oligodendrocytes had previously been determined and characterized in the early 1990s by Dubois-Dalq and her colleagues in a series of elegant studies (Armstrong et al., 1992; Gogate et al., 1994), and these cells were subsequently identified and mapped histologically (Scolding et al., 1998). However, mitotically competent progenitors capable of giving rise to oligodendrocytes were not isolated from adult human brain tissue until later (Roy et al., 1999), when promoter-based sorting permitted the isolation of rare or otherwise hard-to-distinguish progenitor cells from native tissues. In this approach, fluorescent reporters such as green fluorescent protein (GFP) are placed under the control of promoters for genes selectively expressed in the progenitor cells of interest (Wang et al., 1998). The chimeric promoter-driven GFP transgenes are then either transfected or infected into the cell population containing the target progenitor cell, and, upon GFP expression by the cells of interest, the progenitor pool is then extracted by fluorescence-activated cell sorting (FACS). This approach has allowed the identification and isolation of rare neural progenitor cell populations from the ventricular zone and hippocampus, from both fetal and

The first two authors contributed equally to this study.

Contract grant sponsor: NIH/NINDS; Contract grant number: R01NS39559; Contract grant number: R37/R01NS29813; Contract grant number: R01NS33106; Contract grant sponsor: National MS Society; Contract grant sponsor: Christopher Reeve Paralysis Foundation; Contract grant sponsor: Project ALS.

*Correspondence to: Steven A. Goldman, MD, PhD, Department of Neurology and Neuroscience, Cornell University Medical Center, 1300 York Ave., Rm. E607, New York, NY 10021.
E-mail: sgoldm@mail.med.cornell.edu

Received 5 May 2002; Revised 13 June 2002; Accepted 15 June 2002

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jnr.10397

adult human samples (Wang et al., 1998, 2000; Roy et al., 2000a,b; Keyoung et al., 2001).

To determine whether adult human white matter might harbor oligodendrocyte progenitor cells, we constructed plasmid vectors containing the early promoter for the oligodendrocyte protein cyclic nucleotide phosphodiesterase, placed 5′ to the coding region for human GFP (hGFP). When we transfected this construct into dissociates of the adult human capsular white matter, we observed that P/CNP2:hGFP was expressed initially by only a single, morphologically and antigenically discrete class of bipolar cells (Roy et al., 1999). These cells were mitotically competent, in that they incorporated bromodeoxyuridine (BrdU) and continued to proliferate in low-serum base medium containing fibroblast growth factor-2 (FGF2), platelet-derived growth factor (PDGF), and neurotrophin-3 (NT-3). Among the P/CNP2:hGFP$^+$ cells, most initially expressed the early oligodendrocytic marker A2B5 but failed to express the more differentiated markers O4, O1, or galactocerebroside. Some expressed astrocytic glial fibrillary acidic protein (GFAP), but none expressed neuronal markers when identified by their GFP fluorescence in mixed, unsorted cultures. When FACS was used to purify these P/CNP2:hGFP$^+$ cells, most were found to mature as oligodendrocytes, progressing through a stereotypic sequence of A2B5, O4, O1, and galactocerebroside expression (Roy et al., 1999), as during development (Noble, 1997). However, occasional neurons were also noted to arise from these cells, particularly in low-density preparations following high-purity FACS, a condition under which sorted progenitors are largely devoid of autocrine and paracrine growth factors. Thus, the nominally glial progenitor of the adult white matter might actually represent a multipotential neural progenitor cell, restricted to the glial and oligodendrocytic lineage by the local white matter environment. As a result, we have designated these cells *white matter progenitor cells* (WMPCs), rather than simply glial progenitors, in recognition of their intrinsically broad lineage potential. Importantly, these cells are not rare: By cytometry based on P/CNP2-driven GFP, WMPCs made up over 0.4% of the sorted white matter cell pool (Roy et al., 1999). With correction for an average plasmid transfection efficiency of 13%, over 3% of dissociated white matter cells might be competent to serve as progenitor cells.

In the present study, we sought to assess the engraftability and myelinogenic competence of these cells when introduced into a region of central demyelination. To this end, we employed a lysolecithin model, in which we injected human WMPCs into the lesioned adult rat brain and then assessed the integration, phenotypic maturation, and myelinogenic competence of the implanted human cells in the environment of the lesioned adult rodent white matter. In addition, we sought to achieve a higher yield means of separating these progenitor cells from surgical samples of the adult white matter, to increase the feasibility of using them in experimental transplantation.

MATERIALS AND METHODS

Adult Human White Matter Dissociation and Culture

Surgically resected adult human brain tissue samples were obtained from five patients ranging from 22 to 49 years old (two males with aneurysms, a female with a geographically distant and histologically circumscribed hemangioma, a female with temporal lobe epilepsy, and a male with an arteriovenous malformation). Surgical resections of forebrain white matter were collected in Ca^{2+}/Mg^{2+}-free Hank's balanced salt solution (HBSS), minced, rinsed twice in PIPES (in mM: 120 NaCl, 5 KCl, 25 glucose, and 20 PIPES), and digested in papain-PIPES (11.4 U/ml papain; Worthington, Freehold, NJ) and DNase I (10 U/ml; Sigma, St. Louis, MO) on a rocker at 37°C for 1.5 hr. The cells were collected by centrifugation at 200*g* in an IEC Centra-4B centrifuge, resuspended in Dulbecco's modified Eagle's medium (DMEM)/F-12/N2 with DNase I (10 U/ml), and incubated at 37°C for 30 min. The samples were spun again and the pellets recovered in 2 ml of DMEM/F12/N2. The cells were then dissociated by sequentially triturating for 20, 10, and 5 times, respectively, through Pasteur pipettes fire polished to decreasing bore diameters. Undissociated pieces were removed by passage through a 40 μm mesh. The cells were collected and rinsed with DMEM/F-12/N2 containing 10% plasma-derived fetal bovine serum (PD-FBS; Cocalico, Reamstown, PA) to stop the dissociation. The cells were then suspended in DMEM/F12/N2 containing PDGF-AA (20 ng/ml; Sigma), FGF-2 (10 ng/ml; Sigma), and NT-3 (2 ng/ml; Regeneron, Tarrytown, NY) and plated in 100 mm uncoated petri dishes (Corning, Corning, NY).

Magnetic Separation of A2B5$^+$ Cells

After 48 hr in culture, cells dissociated from adult human white matter were collected by washing the plates with Ca^{2+}/Mg^{2+}-free HBSS. The total number of viable cells was determined using calcein (Molecular Probes, Eugene, OR). The cells were incubated with supernatant of hybridoma cells expressing the monoclonal IgM antibody A2B5 (clone 105; American Type Culture Collection, Manassas, VA). Incubation proceeded for 30–45 min at 4°C on a shaker. The cells were washed three times with 10 times the labeling volume in phosphate buffer containing 0.5% bovine serum albumin (BSA) and 2 mM EDTA. The cells were incubated with 1:4 microbead-tagged rat anti-mouse IgM (Miltenyi Biotech) for 30 min at 4°C on a shaker. For flow cytometric analysis, some cells were incubated for the same duration with fluorescein isothiocyanate (FITC)-tagged goat anti-mouse IgM (1:50). The cells were washed three times and resuspended in an appropriate volume of buffer. The A2B5$^+$ cells were separated using positive selection columns, type MS$^+$/RS$^+$ or LS$^+$/VS$^+$ (MACS; Miltenyi Biotech).

Labeling of Human Donor Progenitor Cells

Lipophilic dye tagging. Some A2B5-sorted cells were tagged with 1,1′-dihexadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI; Molecular Probes) at 0.01 mg/ml. DiI-tagged cells were noted to retain their fluorescence discretely for at least 1 week after tagging and transplantation. For longer survival times, cells were identified as donor derived and imaged on the basis of anti-human nuclear antibody immunostaining or by BrdU tagging in vitro prior to implantation, with

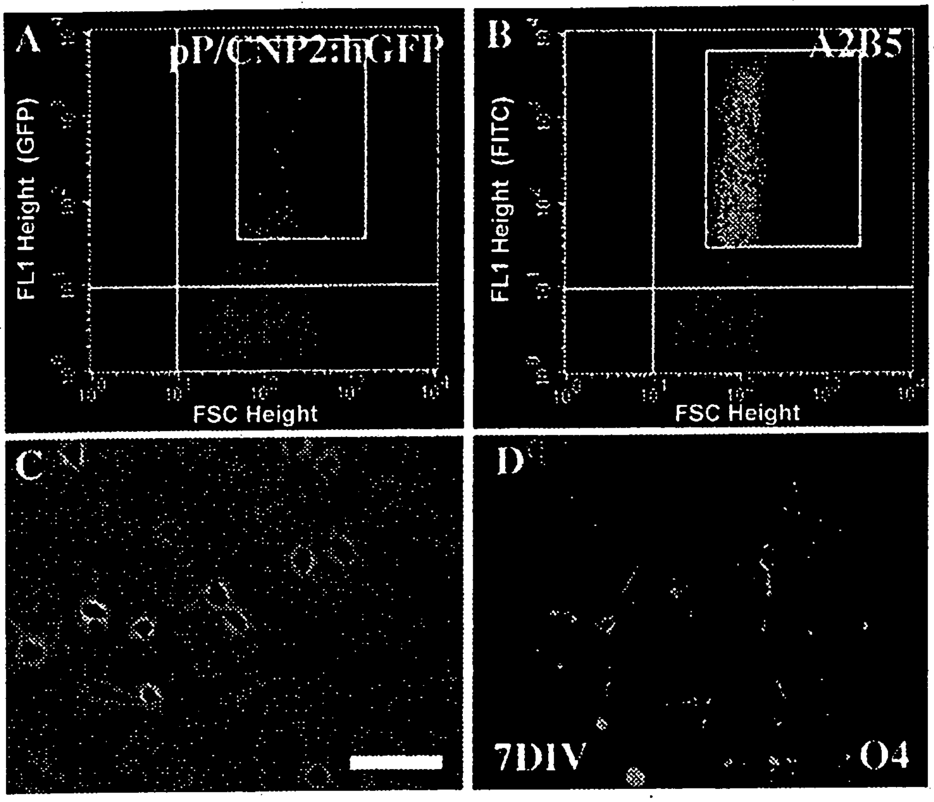

Fig. 1. Oligodendrocyte progenitor cells may be extracted from the adult human white matter. **A,B:** Separation plots of progenitor cells derived from dissociates of adult human white matter. These graphs plot forward scatter (FCS), an index of cell size, against fluorescence intensity (FL1). A shows separation of progenitors based on CNP2:hGFP, and B exhibits FACS separation based on A2B5-immunoreactivity. The two cell populations sort into homologous quadrants on forward scatter, indicating that the two methods extract cells of the same size. They also sort into identical pools as defined by side scatter (not shown), indicating that their shape and internal reflectance are analogous as well. **C,D:** By 7 days in culture, most of the A2B5-sorted population had become oligodendrocytes (C, phase; D, fluorescence). This was manifested in their expression of O4 immunoreactivity (green), which recognizes a sulfatide epitope characteristic of oligodendrocytes. Scale bar = 40 μm.

subsequent BrdU immunodetection. Alternatively, some human donor progenitors were genetically tagged with GFP, using lentiviral delivery.

Lentiviral GFP. In some experiments, the A2B5-sorted cells were infected 24 hr after separation with a purified VSV-pseudotyped lentivirus (1×10^5/ml) constructed to express enhanced GFP (EGFP) under the control of the cytomegalovirus (CMV) promoter, with a WPRE 5 woodchuck posttranscription regulatory element. The latter acts like poly-A by stabilizing the transcript without stopping transcription. The lentivirus was generated by cotransfecting plasmids pCMV-D-R8.91, pMD.G, and pHRCMVGFPwsin into 293T cells (Han et al., 1999). Viral particles were collected after 72 hr. The viruses were partially purified by centrifuging the collected supernatant at 60,000*g* for 2 hr. The sorted A2B5 cells were infected with the virus in the presence of polybrene (8 μg/ml). GFP expression was typically observed by over half of the cells within each infected cell culture within 24 hr after infection. The cells were harvested for transplantation 48 hr after viral infection.

Surgery

Lysolecithin injection. Lesions were produced in the corpus callosa of 200–225 g male rats by stereotaxic bilateral injection of 1 μl of either 1.5% (used for xenografts of GFP-tagged cells only) or 2% lysolecithin type V (Sigma). The coordinates were 1.1 mm posterior to Bregma, 1.0 mm lateral to the midline, and 2.8 mm ventral (Gensert and Goldman, 1997), and the myelinotoxin was delivered at 20 μl/hr.

Transplantation. Three days after lysolecithin instillation, 100,000 A2B5-sorted cells were delivered in 2 μl HBSS into the site of lysolecithin injection, by infusion over 3 min (20 μl/hr). Control animals received comparable injections of saline. Animals were sacrificed at 1 (n = 2), 2 (n = 2), 3 (n = 3), 4 (n = 3), and 8 (n = 1) weeks after xenograft.

Immunosuppression

All animals were immunosuppressed with cyclosporin (Sandimmune; Novartis; 50 mg/ml). Animals received 15 mg/kg daily, beginning on the day of lysolecithin lesion and proceeding daily thereafter until sacrifice. Animals receiving GFP-tagged xenografts were given 20 mg/kg.

Immunohistochemistry

Animals were perfused via intracardiac catheter with HBSS with Ca^{2+}/Mg^{2+}, followed by 4% paraformaldehyde, with postfixation for several hours in 4% paraformaldehyde, passage through increasing concentrations of sucrose to 30%, and freezing during embedding in OCT (Lipshaw). The brains were then cut in 15 μm sections on a Hacker cryostat. Sections were processed for one or more of the following antigenic markers: anti-human nuclear protein (Chemicon, Temecula, CA; MAb1281; 1:50 for 2 days, 4°C), anti-CNP (Sternberger, Baltimore, MD; MAb SMI-91; 1:1,000 overnight, 4°C), anti-human GFAP (SMI 21; 1:1,000, overnight, 4°C), or antimyelin basic protein (anti-MBP; Chemicon Ab980; 1:100 overnight, 4°C). Secondary antibodies included FITC, Cy5, and Texas red-tagged anti-mouse IgM and IgG, anti-rabbit IgG (Jackson

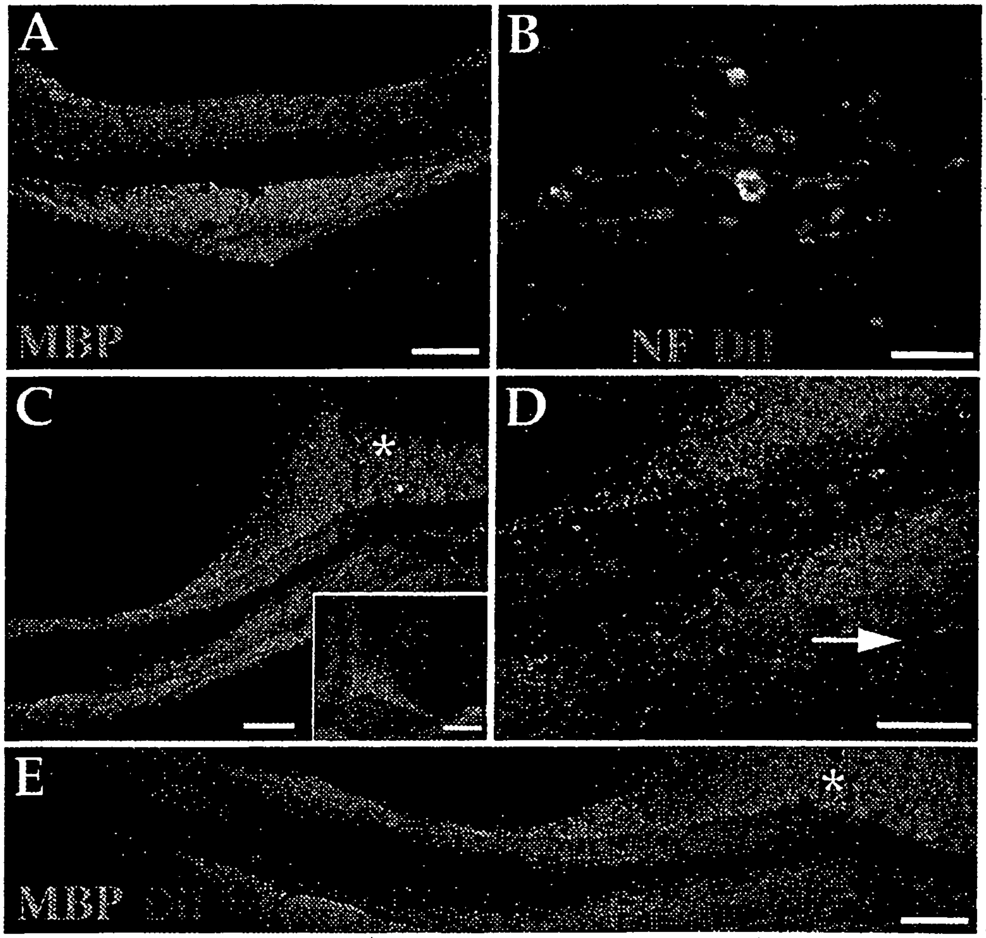

Fig. 2. Implanted white matter progenitors migrated widely throughout the demyelinated callosum. **A–E:** Sorted adult human white matter progenitors were transplanted into lysolecithin-induced demyelinated lesions in the corpus callosa of adult rats. A shows that lysolecithin infusion yielded demyelinated plaques in the subcortical white matter. This rat was injected with 1 μl of 2% lysolecithin-V, directly into the central core of the corpus callosum, then sacrificed for histology 1 week later and immunostained for myelin basic protein (MBP). The large central lesion is visible as the discoid region of MBP immunonegativity, surrounded by the otherwise MBP$^+$ callosum (green). B: Neurofilament$^+$ axons (green) initially survived lysolecithin lesion, as seen here 1 week after lesion of the callosum. MBP immunoreactivity (green) has been lost from this lesion core, and implanted progenitors have just immigrated to the lesion (orange). However, axonal spheroids were frequent within the lysolecithin lesion bed, indicating some degree of early injury and transection, to which spheroid formation is a response. The ability of implanted progenitors to effect repair is thus limited by the viability and integrity of the targeted axonal cohort. C: DiI-labeled human progenitor cells (red) 1 week after implant. Even at this early time point, the cells extend throughout the demyelinated lesion, which is characterized by its lack of MBP immunoreactivity (green). The cannula track (*) indicates the site of cell injection into the demyelinated lesion, which was induced 3 days before 10^5 sorted, DiI-tagged (red) human progenitors were delivered in 2 μl. **Inset:** Fluorescent microbeads (red) injected into regions of lysolecithin demyelination (MBP; green) failed to disperse beyond their site of injection. D: The transplanted cells migrated throughout the demyelinated plaque, but not beyond its borders, except for occasional migrants that followed the parenchymal surfaces of blood vessels (arrow). The restriction of migration to demyelinated regions suggests that normal myelin impeded the migration of these cells (bottom). E: This low-power montage illustrates the extent and rapidity of migration by engrafted white matter progenitors. Within 1 week of implantation into this demyelinated callosum, the cells traversed the midline to infiltrate the lesion bed in the contralateral hemisphere. The longitudinal extent of this lesion is approximately 6 mm, and the rat was sacrificed 1 week after implantation. Scale bars = 200 μm in A,E; 20 μm in B; 100 μm in C,D; 500 μm in inset.

Immunoresearch, West Grove, PA), and Alexa 488- and 594-tagged anti-mouse and anti-rabbit IgG (Molecular Probes).

Imaging

Brain sections were photographed using an Olympus Fluoview confocal coupled to an IX70 photomicroscope. Images were acquired in both red and green emission channels using an argon-krypton laser, as previously described (Benraiss et al., 2001). The images were then viewed as stacked z-dimension images, both as series of single 0.5 μm optical sections and as merged images thereof. The z-dimension reconstructions were all observed in profile; every human cell doubly labeled with a

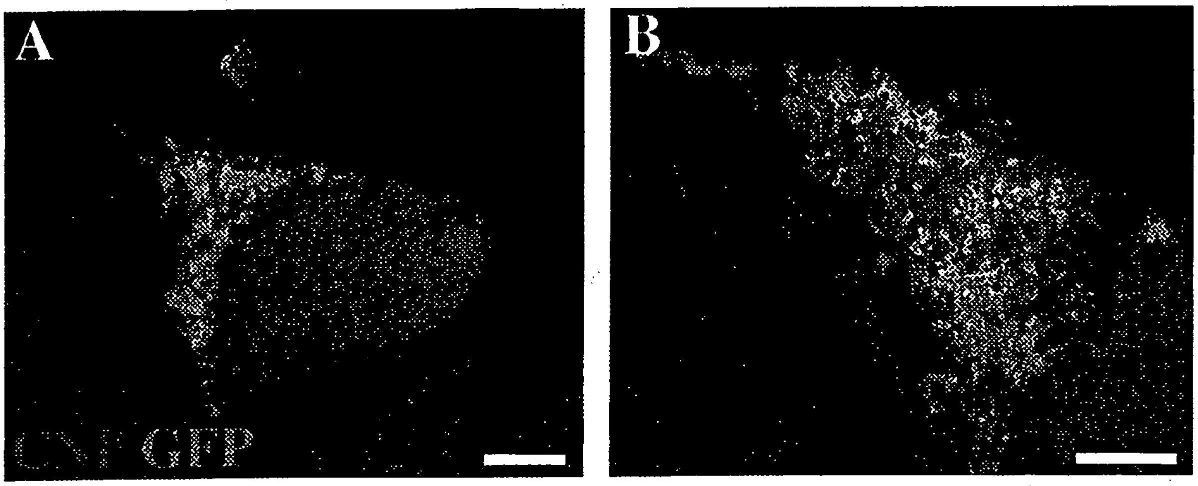

Fig. 3. Normal white matter was nonpermissive for the migration of adult progenitors. A2B5-sorted human progenitors xenografted into the normal adult rat brain failed to migrate beyond the injection site. For this figure, the human cells were prelabeled in vitro with lentiviral GFP (green) to allow the implanted progenitors and their progeny to be identified. **A,B:** The implanted WMPCs shown in two different fields here were imaged in an animal killed 4 weeks after implantation. The implanted cells did not migrate beyond the borders of the initial injectate. This contrasted with the widespread migration of these cells throughout demyelinated foci, as in Figure 2. Scale bars = 200 μm in A; 100 μm in B.

phenotypic marker was observed orthogonally in both the vertical and the horizontal planes to ensure double labeling.

RESULTS

Olig dendrocyte Progenitors of the Human White Matter Are Selected by A2B5 Expression

In previous studies, we found that transduction of adult human white matter dissociates with plasmids bearing the early oligodendrocytic promoter P/CNP2, driving the fluorescent reporter hGFP, permitted FACS of oligodendrocyte progenitor cells from cultured brain tissue (Roy et al., 1999). Because P/CNP2:hGFP$^+$ cells typically expressed A2B5 immunoreactivity, we asked whether separation based on A2B5 might yield the same pool of mitotic oligodendrocyte progenitor cells. To this end, we first used FACS based on A2B5 expression to extract A2B5$^+$ cells from adult WM dissociates (Fig. 1). We found that 2.7% ± 0.4% of the cells could be separated as A2B5$^+$ (n = 5 patients). This compared with P/CNP2: hGFP-based FACS, from which 0.59% ± 0.1% of the cells could be sorted as P/CNP2:hGFP$^+$; the mean transfection efficiency of 13.5% would have predicted that as many as 4.4% of sorted WM cells were potentially P/CNP2: hGFP$^+$. Using that figure as an arbitrary benchmark, we can estimate that A2B5-based FACS achieved the viable extraction of 57.4% (=2.7/4.4 × 100) of the P/CNP2: hGFP-predicted progenitor cells in the adult white matter.

We next used, based on this figure, immunomagnetic sorting (IMS) to select A2B5$^+$ cells from adult WM dissociates. IMS permits a higher yield than FACS, with a greater recovery and higher viability achieved at the expense of a higher incidence of false positives. We found that, by IMS, 2.87% ± 0.7% of the cells were separated as A2B5$^+$ (n = 3 patients). This was in accordance with the incidence of WMPCs estimated by both P/CNP2:GFP-based FACS and A2B5-based FACS.

As with the P/CNP2:hGFP$^+$ cells, the A2B5$^+$ cells were mitotic and gave rise largely to oligodendrocytes (Fig. 2). When exposed to BrdU for the first 2 days after sorting, A2B5-defined cells incorporated the label and expanded in number, indicating their persistent replication in vitro. Over the week thereafter, most began to express definitive markers of the oligodendrocytic phenotype; by 1 week after isolation, >70% expressed the oligodendrocytic antigen O4. Together, these data indicate that A2B5-based FACS and IMS of the adult human white matter yields a population of oligodendrocyte progenitor cells that may be homologous to that recognized by P/CNP2: GFP-based isolation and FACS.

Lysolecithin Lesions Provide Demyelinated Foci Appropriate for Experimental Implantation

To establish whether adult human WMPCs could survive xenograft to adult brain parenchyma, we implanted human WMPCs into lysolecithin-demyelinated callosal lesions in adult rats. Lysolecithin is a useful agent for achieving predictable, focal lesions of the white matter (Gensert and Goldman, 1997). It results in local demyelination with local oligodendrocytic loss, some axonal loss, and relative preservation of astrocytic and endothelial elements. Spontaneous remyelination may occur following lysolecithin lesioning and follows a time course that is dependent on the type of lysolecithin (Sedal et al., 1992), its volume and concentration (Woodruff and Franklin, 1999), and the age of the animals (Shields et al., 1999).

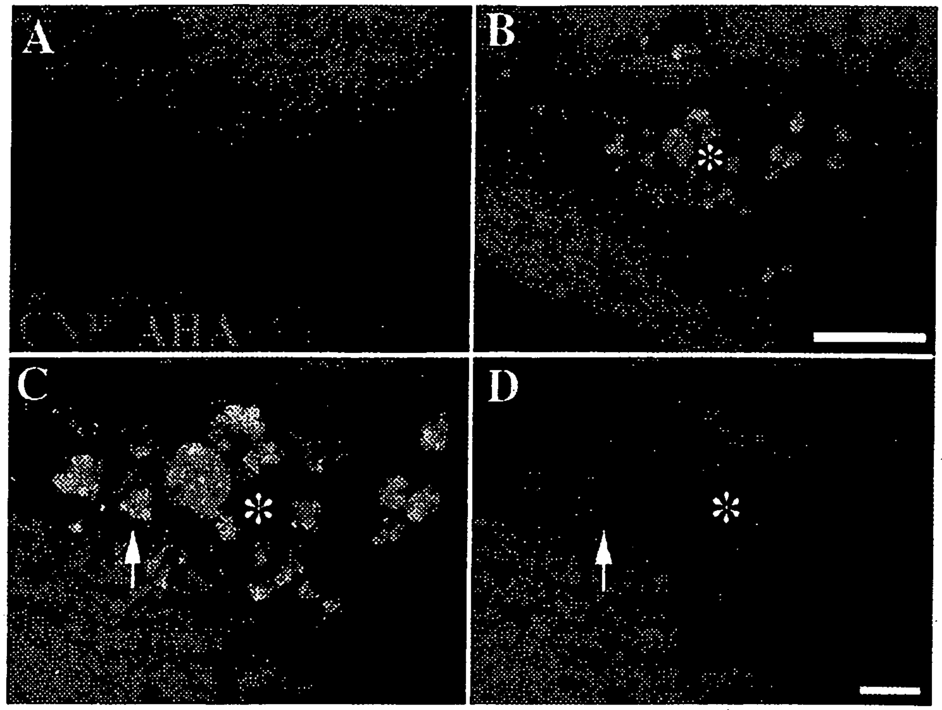

Fig. 4. Xenografted adult white matter progenitors become oligodendrocytes and astrocytes. Implanted A2B5-sorted progenitors typically developed expression of oligodendrocytic CNP protein within 2 weeks of implantation. **A:** In a control animal that received a saline injection, CNP protein (red) remains absent from the demyelinated central core of the callosum (black) 15 days after lesion. In the matched animal shown in **B**, human white matter progenitor cells, recognized by anti-human nuclear antibody (AHA; green), were noted both to fill in the lesion and to express oligodendrocytic CNP (red) 15 days after implantation. **C** shows a higher magnification of this field; **D** demonstrates CNP expression (red) associated with the cell bodies of the human cells visualized as AHA$^+$ in B. Asterisks are located in the same position; arrows in C and D indicate the same cell. Scale bar = 20 μm in B (for A,B); 5 μm in D (for C,D).

Therefore, lysolecithin lesions mimic salient aspects of acute inflammatory demyelination.

We first confirmed the prior observations of Gensert and Goldman (1997) that lysolecithin lesion was associated with a focal lesion of capsular myelin, with initial injury predominantly limited to oligodendrocytes and their myelin. When assessed 1 and 3 weeks after 1 μl injections of 2% lysolecithin-V, these lesions exhibited a mild degree of reactive astrocytosis within the demyelinated focus, the vascular architecture of which appeared intact. No myelin could be visualized by staining for MBP within 2 mm of the callosal injection site. In addition, oligodendrocytes were markedly diminished, with a >95% loss of CNP$^+$ cells within the MBP-demarcated lesion (Fig. 2A). Axons were present, as assessed by neurofilament staining, but axonal spheroids were common, indicating some degree of axonal damage and early loss (Fig. 2B).

Adult Human-Derived A2B5-D fined Progenitors Survive and Rapidly Migrate Upon Xenograft to Lysolecithin-Demyelinated Foci of the Adult Rat Brain

We next prepared A2B5-sorted progenitor cell pools from adult human white matter and stereotaxically implanted them into both normal and lysolecithin-lesioned adult rat brain. A2B5$^+$ cells (1×10^5) were implanted into each lesion bed 3 days after a 1 μl injection of 2% lysolecithin. Some donor cells were prelabeled with the lipophilic tracking dye PKH26 to allow their detection after implantation (Horan and Slezak, 1989). Other donor cells were instead localized using human-specific donor cell antigens. At 1, 2, 3, 4, and 8 weeks after implantation, the recipient brains were fixed and prepared for histologic analysis.

The implanted cells migrated rapidly, throughout the extent of the demyelinated lesions. Within 1 week of implantation, the cells readily traversed the midline to infiltrate the farthest reaches of the demyelinated lesion beds, which often extended over 6 mm in breadth. The migration rate of the cells was hence roughly 1 mm/day, or almost 50 μm/hr within the lesion (Fig. 2B–D).

The surprisingly rapid and extensive migration of the implanted cells raised the possibility that the initial pressure of the injection was contributing to their local dispersal. To ensure that implanted donor progenitor cells were not infiltrating their target lesions as a function of hydraulic pressure, we slowly infused them in 2 μl over 3 min. As an

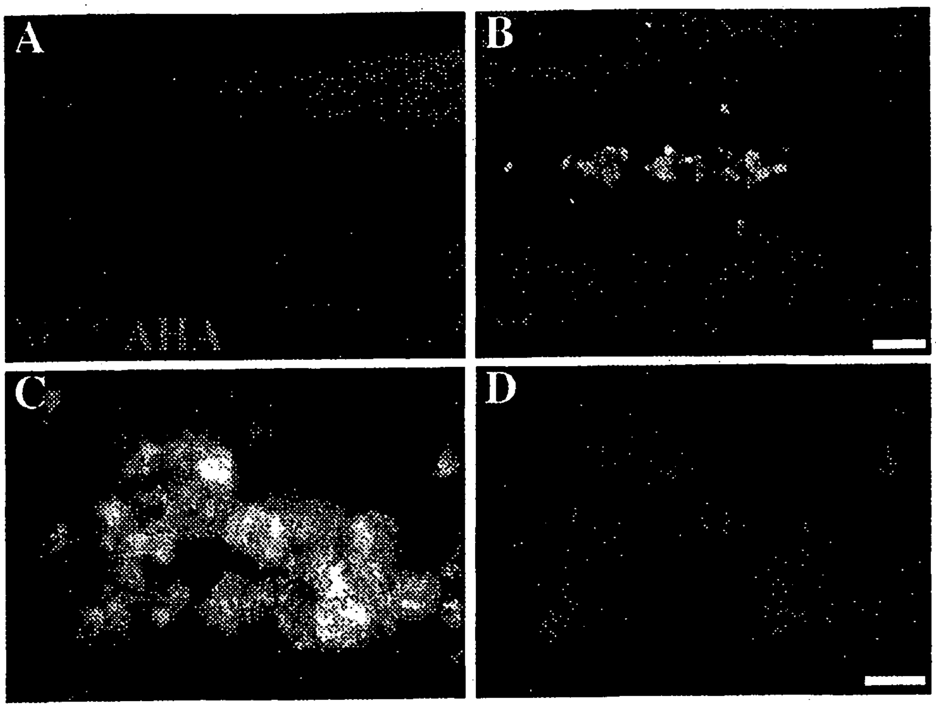

Fig. 5. White matter progenitors express MBP after engraftment to demyelinated foci. Sorted oligodendrocyte progenitor cells derived from the adult human white matter were injected into adult lysolecithin-lesioned rat corpus callosum and were noted to express MBP within 3 weeks thereafter. **A:** In a control animal that received a saline injection, MBP (red) remains absent from the demyelinated central core of the callosum (black) 3 weeks after lesion. **B:** In a rat that received 100,000 sorted progenitor cells unilaterally, human nuclei (green) are surrounded by MBP (red) in the center of the lesion 21 days after implantation (24 days after lysolecithin injection). **C,D:** High-power image showing a cluster of AHA$^+$ human cells (green) associated with a plethora of MBP$^+$, myelinating, oligodendrocytic membranes (red). D focuses on the MBP$^+$ membranes of this field; the oligodendrocytic lamellopodia (red) appear in various stages of ensheathment. Scale bar = 20 μm in B (for A,B); = 5 μm in D (for C,D).

additional control, two rats were injected with 6 μm fluorescent microbeads (Becton-Dickinson, San Jose, CA; 488 nm excitation). In total 100,000 beads (2 μl/3 min) were delivered into callosal foci of lysolecithin demyelination 3 days after lysolecithin injection. The rats were sacrificed 2 hr after surgery, and their brains were cryosectioned and immunostained for MBP. The microbeads were noted to line the cannula track and otherwise remained within a focal deposit at the injection site (Fig. 2C). These findings strongly suggested that pressure injection per se was not associated with significant mechanical dispersion of injected cells.

Normal Brain Is Nonpermissive for Migration of Adult Oligodendrocyte Progenitor Cells

Despite the rapid migration of the implanted progenitor cells throughout the lesion beds (Fig. 2B,C), the cells were typically restricted to regions of demyelination, rarely extending into normal surrounding myelin. Even the few cells that were typically noted to have infiltrated normal myelin appeared to have migrated therein along the extraluminal surfaces of blood vessels. The latter appeared to be limited to vessels that at some point traversed the lesion bed and thereby presented their adventitial surfaces to the migrating implanted progenitors.

On this basis, we asked whether the restriction of implanted progenitors to the lesion site reflected a relative preference for the implanted progenitors to the demyelinated lesion site or whether it instead reflected an absolute impediment of normal white matter to progenitor migration (Jefferson et al., 1997). To this end, we genetically tagged A2B5-sorted adult human WMPCs with CMV-driven EGFP by infecting them in vitro with a lentiviral GFP vector (see Materials and Methods). The fluorescent human WMPCs were then implanted into the intact subcortical white matter of four adult rats to loci including the callosum, hippocampal commissure, and stria medullaris. The fate of the tagged cells was then assessed by sacrificing three of the animals 1 month after implantation and one rat at 2 months. We found that, when injected into intact white matter, the adult progenitor cells remained localized to the implant sites: Whether assessed 4 or 8 weeks after implantation, the cells migrated no farther than the bounds of the initial injectates (Fig. 3).

Adult White Matter Pr genitors Differentiate as MBP$^+$ Oligodendrocytes Upon Xen grafting

The engraftment sites each harbored substantial populations of viable cells, many of which expressed CNP protein, indicating their oligodendrocytic maturation.

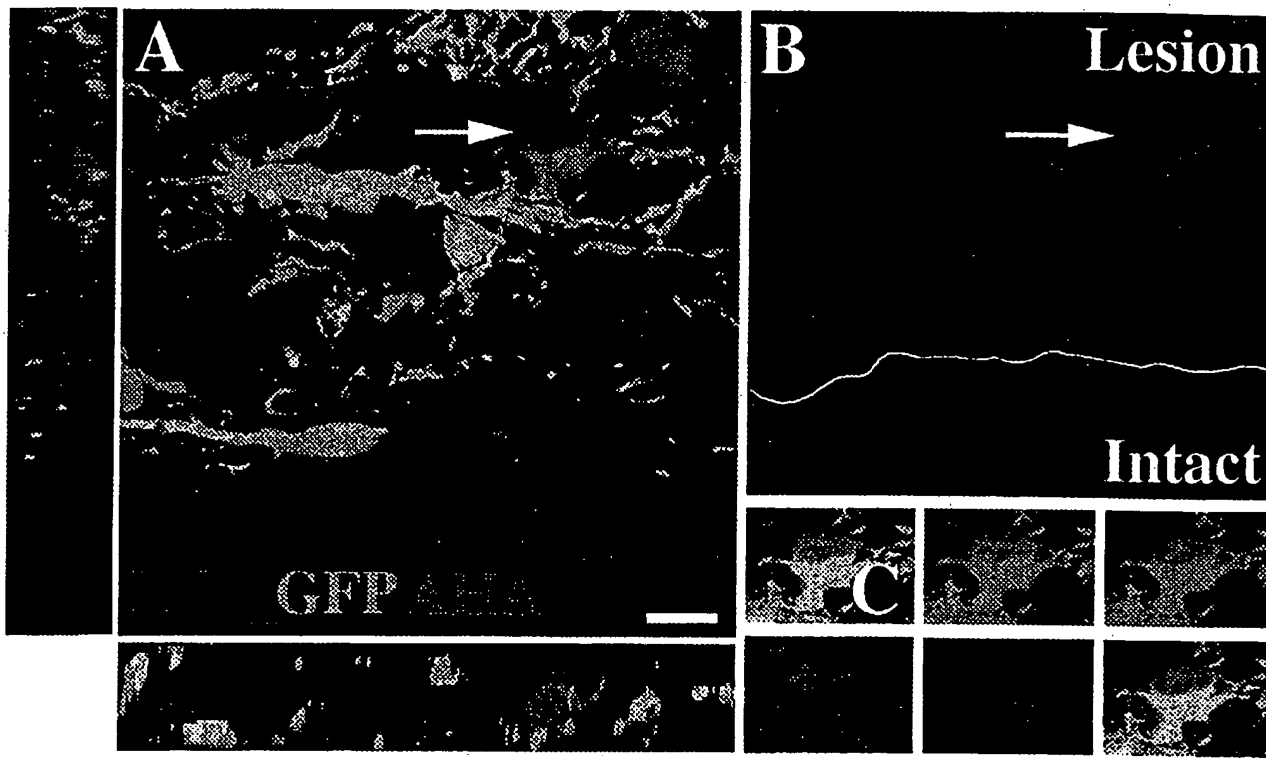

Fig. 6. Genetically tagged adult human-derived white matter progenitors can mature and survive after xenografting. Sorted white matter progenitor cells were tagged with lentivirally delivered GFP, then injected into the lysolecithin-lesioned rat corpus callosum. The recipient animal was killed and its brain immunostained and imaged by confocal microscopy 8 weeks after cell implantation. In A, the GFP$^+$ human progenitors (green) are seen to express human nuclear antigen (AHA; orange), confirming the stability and donor cell restriction of the tag. The side and bottom panels appended to A show orthogonal side views taken through the indicated MBP$^+$ human donor-derived cell (blue/green; arrow). B: Blue color channel of A shows that several of the GFP-tagged human progenitors implanted into the lesion site have differentiated as MBP$^+$ oligodendroglia (line demarcates lesion border). Many have also matured as astrocytes (data not shown). C shows the cell indicated in A, emphasizing its coexpression of lentiviral GFP, human nuclear antigen (AHA), and MBP. Scale bar = 20 μm.

CNP expression typically appeared in implanted adult A2B5-sorted progenitors within 2 weeks of implantation (Fig. 4). By 3 weeks, many had developed expression of MBP (Fig. 5). These human donor-derived cells were noted to project MBP$^+$ lamellopodia; at low magnification, they were associated with a fine, filamentous array of myelinating fibers. These observations suggested the initiation of progenitor-associated myelinogenesis within the lesion site (Fig. 5). With cyclosporin immunosuppression, we found that these cells could survive for at least 2 months in lysolecithin-demyelinated rat recipients (Figs. 6, 7).

To visualize better the expression of myelin-associated antigens by implanted human progenitors, we also implanted four lysolecithin-lesion animals with lentiviral GFP-tagged human WMPCs. These animals were sacrificed after 4 or 8 weeks, and the fate of the tagged progenitors was assessed histologically. At both time points, GFP-tagged cells were found to have differentiated as admixed populations of oligodendrocytes and astrocytes. No neurons were noted to have arisen from these engrafted progenitors in a matched set of βIII-tubulin-immunostained sections (not shown). Within the lesions, many MBP$^+$ oligodendrocytes were noted to be GFP tagged and, hence, derived from donor human progenitor cells (Fig. 6). At the lesion borders, a preponderance of GFAP$^+$ GFP-tagged cells was typically noted, indicating the astrocytic differentiation of many of the implanted progenitors (Fig. 7). Few cells of either type migrated beyond the lesion borders.

DISCUSSION

Our previous studies revealed the existence of a distinct population of mitotic oligodendrocyte progenitor cells in the adult human subcortical white matter. These cells are present in both sexes and into senescence and are both ubiquitous and relatively abundant in the adult forebrain white matter. In this study, we found that these oligodendrocyte progenitor cells of the adult human subcortical white matter may also be identified and isolated on the basis of their surface expression of the epitope recognized by MAb A2B5 and that this antigenic phenotype includes those cells defined by CNP2-driven GFP. IMS based on A2B5 expression has allowed us to extract these

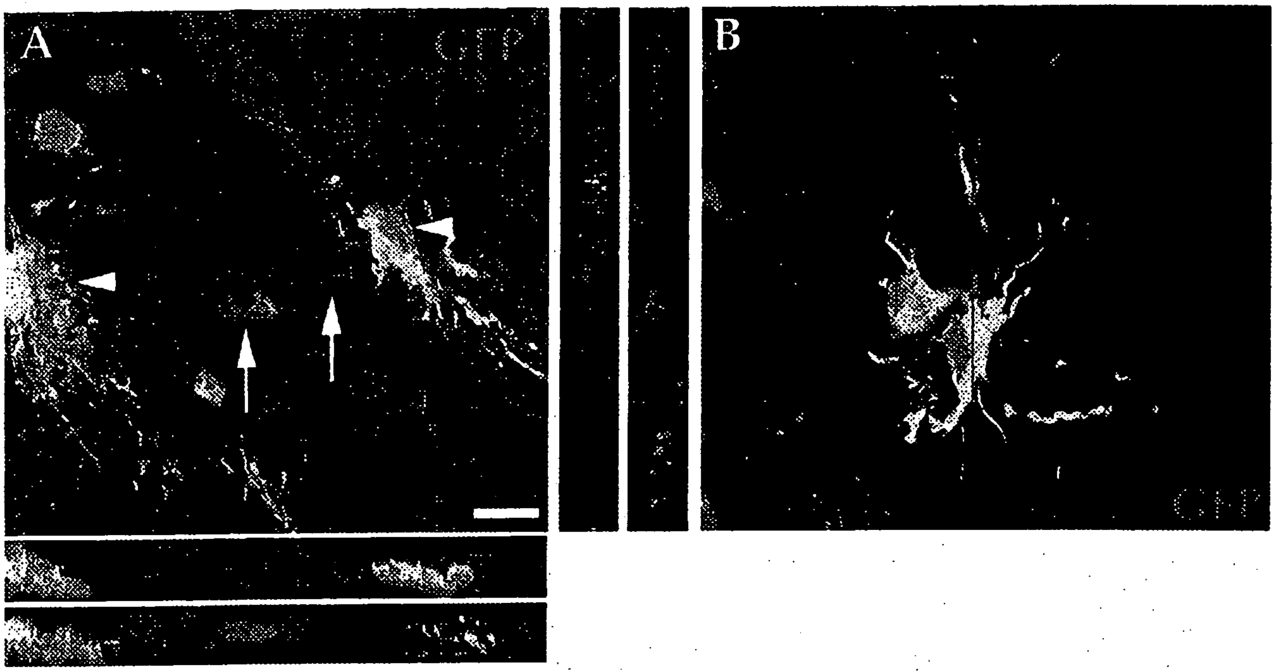

Fig. 7. Both astrocytes and oligodendrocytes arose from implanted adult human white matter progenitors. In **A**, a confocal composite shows two GFP-tagged human (green) MBP^+ (red) oligodendrocytes in the lesion bed of a lysolecithin-injected rat callosum 8 weeks after cell implantation. In addition to the MBP^+ cells (arrows), other human progenitor-derived cells were also present that did not express MBP and that instead manifested astrocytic morphologies (arrowheads). In **B**, immunolabeling for human GFAP (red) revealed that many of the GFP-tagged human progenitors had in fact given rise to astrocytes. Scale bar = 20 μm.

cells from human white matter dissociates in numbers sufficient for experimental xenografting.

The implanted white matter progenitors were found to be highly motile and migratory, infiltrating the demyelinated regions of the white matter over distances up to 1 cm in the week after implantation. However, they were found to avoid normal myelin, which effectively excluded their infiltration. The failure of WMPCs implanted into the normal brain to migrate beyond the injection bed stood in sharp contrast to the fate of otherwise identical cells implanted into lesioned white matter, which migrated rapidly and efficiently throughout the available demyelinated lesion bed. These observations suggest that normal adult white matter is nonpermissive for the migration of adult-derived WMPCs. This restriction appears to be stringent and does not merely reflect demyelinated tissue acting as a preferential substrate for progenitor migration. In a general sense, progenitor cells may be subject to the same types of negative influences on their migration as are axons, whose extension is suppressed in the environment of normal white matter (GrandPre et al., 2000; Chen et al., 2000). However, whereas several myelin-associated moieties that suppress axonal extension, and the axonal receptors for these repulsive ligands, have been identified (Fournier et al., 2001), the operative white matter signals that restrict progenitor cell migration have yet to be determined. The characterization of these repulsive ligands and of their anticipated progenitor cell receptors will likely constitute an important avenue for future study.

The engrafted adult-derived progenitors differentiated largely as oligodendrocytes, and also as astrocytes, and exhibited myelin protein expression in regions of experimental demyelination. The time course of this process was relatively rapid; oligodendrocytic differentiation, as reflected by CNP protein expression, ensued within 2 weeks of donor cell isolation and implantation. Myelinogenesis appeared to follow closely, such that MBP expression attributable to donor cells was evident within 3 weeks of implantation. The efficiency of myelination was difficult to assess in this study, insofar as we did not systematically assess the persistence of axons in these lesions. This caveat notwithstanding, our observations suggest that the introduction of highly enriched preparations of progenitor cells derived from the adult human white matter may permit the structural repair of demyelinated lesions in the adult CNS.

ACKNOWLEDGMENT

We thank Dr. Didier Trono for the lentiviral plasmids and protocols.

REFERENCES

Armstrong RC, Dorn HH, Kufta CV, Friedman E, Dubois-Dalcq ME. 1992. Pre-oligodendrocytes from adult human CNS. J Neurosci 12: 1538–1547.

Benraiss A, Chmielnicki E, Roh D, Goldman SA. 2001. Adenoviral BDNF induces both neostriatal and olfactory neuronal recruitment from endogenous progenitor cells in the adult forebrain. J Neurosci 21:6718–6731.

Chen M, Huber, A., vander Haar M, Frank M, Schnell L, Spillman A, Christ F, Schwab ME. 2000. Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature 403:434–439.

Fournier A, GrandPre T, Strittmatter SM. 2001. Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. Nature 409:341–346.

Gensert JM, Goldman JE. 1996. In vivo characterization of endogenous proliferating cells in adult rat subcortical white matter. Glia 17:39–51.

Gensert JM, Goldman JE. 1997. Endogenous progenitors remyelinate demyelinated axons in the adult CNS. Neuron 19:197–203.

Gogate N, Verma L, Zhou JM, Milward E, Rusten R, O'Connor M, Kufta C, Kim J, Hudson L, Dubois-Dalcq M. 1994. Plasticity in the adult human oligodendrocyte lineage. J Neurosci 14:4571–87.

Goldman SA. 2001. Neural progenitor cells of the adult human forebrain. In: Rao M, editor. Stem cells and CNS development. Totowa, NJ: Humana. p 177–206.

GrandPre T, Nakamura F, Vartanian T, Strittmatter S. 2000. Identification of the Nogo inhibitor of axon regeneration as a reticulon protein. Nature 403:439–444.

Han J, Mhatre A, Wareing M, Pettis R, Gao W, Zufferey R, Trono D, Lalwani A. 1999. Transgene expression in the guinea pig cochlea mediated by a lentivirus-derived gene transfer vector. Hum Gene Ther 10: 1867–1873.

Horan P, Slezak S. 1989. Stable cell membrane labeling. Nature 340:167–168.

Jefferson S, Jacques T, Kiernan B, Scott-Drew S, Milner R, ffrench-Constant C. 1997. Inhibition of oligodendrocyte precursor motility by oligodendrocyte processes: implications for transplantation-based approaches to multiple sclerosis. Mult Scler 3:162–167.

Keyoung HM, Roy N, Louissant A, Benraiss A, Mori Y, Okano H, Goldman SA. 2001. Specific identification, selection and extraction of neural stem cells from the fetal human brain. Nat Biotechnol 19:843–850.

Levison S, Young G, Goldman JE. 1999. Cycling cells in the adult rat neocortex preferentially generate oligodendroglia. J Neurosci Res 57: 435–446.

Noble M. 1997. The oligodendrocyte-type 2 astrocyte lineage: in vitro and in vivo studies on development, tissue repair and neoplasia. In: Gage YCF, editor. Isolation, characterization and utilization of CNS stem cells. Berlin: Springer-Verlag. p 101–128.

Roy N, Wang S, Benraiss A, Fraser R, Gravel P, Braun P, Goldman SA. 1999. Identification, isolation and promoter-defined separation of mitotic oligodendrocyte progenitor cells from the adult human subcortical white matter. J Neurosci 19:9986–9995.

Roy N, Benraiss A, Wang S, Fraser RAR, Goodman R, Couldwell WT, Nedergaard M, Kawaguchi A, Okano H, Goldman SA. 2000a. Promoter-targeted selection and isolation of neural progenitor cells from the adult human ventricular zone. J Neurosci Res 59:321–331.

Roy NS, Wang S, Jiang L, Kang J, Benraiss A, Harrison-Restelli C, Fraser RA, Couldwell WT, Kawaguchi A, Okano H, Nedergaard M, Goldman SA. 2000b. In vitro neurogenesis by progenitor cells isolated from the adult human hippocampus. Nat Med 6:271–277.

Scolding N, Franklin R, Stevens S, Heldin CH, Compston A, Newcombe J. 1998. Oligodendrocyte progenitors are present in the normal adult human CNS and in the lesions of multiple sclerosis [see comments]. Brain 121:2221–2228.

Sedal L, Jennings KH, Allt G, Ghabriel MN, Harrison MJ. 1992. Influence of fatty acid content of lysophosphatidyl choline on its myelinotoxic properties. Eur Neurol 32:4–10.

Shields SA, Gilson JM, Blakemore WF, Franklin RJ. 1999. Remyelination occurs as extensively but more slowly in old rats compared to young rats following gliotoxin-induced CNS demyelination [erratum appears in Glia 29:102, 2000]. Glia 28:77–83.

Wang S, Wu H, Jiang J, Delohery TM, Isdell F, Goldman SA. 1998. Isolation of neuronal precursors by sorting embryonic forebrain transfected with GFP regulated by the Tα1 tubulin promoter. Nat Biotechnol 16:196–201.

Wang S, Roy N, Benraiss A, Harrison-Restelli C, Goldman SA. 2000. Promoter-based isolation and purification of mitotic neuronal progenitor cells from the adult mammalian ventricular zone. Dev Neurosci 22:167–176.

Wolswijk G, Noble M. 1989. Identification of an adult-specific glial progenitor cell. Development 105:387–400.

Woodruff RH, Franklin RJ. 1999. Demyelination and remyelination of the caudal cerebellar peduncle of adult rats following stereotaxic injections of lysolecithin, ethidium bromide, and complement/antigalactocerebroside: a comparative study. Glia 25:216–28.